

Computer-Aided Design of Nanoceria Structures as Enzyme Mimetic Agents: The Role of Bodily Electrolytes on Maximising Their Activity.

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Abstract

Nanoceria, typically used for ‘clean air’ catalytic converter technologies because of its ability to capture, store and release oxygen is the same material that has the potential to be used in nanomedicine. Specifically, nanoceria can also be used to control oxygen content in cellular environments; as a ‘nanozyme’, nanoceria mimics enzymes by acting as an antioxidant agent. The computational design procedures for predicting active materials for catalytic converters can therefore be used to design active ceria nanozymes. Crucially, the ceria nanomedicine is not a molecule; rather it is a crystal and exploits its unique crystal properties.

Here, we use ab initio and classical computer modelling, together with experiment, to design structures for nanoceria that maximise its nanozymetic activity. We predict that the optimum shape of the nanoparticles should have (truncated) polyhedral or cuboidal morphologies to expose (active) $\text{CeO}_2\{100\}$ surfaces. It should also contain oxygen vacancies and surface hydroxyl species. We also show that the surface structures strongly affects the biological activity of nanoceria. Analogous to catalyst poisoning, phosphorus ‘poisoning’ - the interaction of nanoceria with phosphate, a common bodily electrolyte – emanates from phosphate ions binding strongly to $\text{CeO}_2\{100\}$ surfaces, inhibiting oxygen capture and release and hence its ability to act as a nanozyme. Conversely, phosphate interaction with $\{111\}$ surfaces is weak and therefore these surfaces protect the nanozyme against poisoning.

The atom-level understanding presented here also illuminates catalytic processes and poisoning in ‘clean-air’ or fuel-cell technologies because the mechanism underpinning and exploited in each technology – oxygen capture, storage and release – is identical.

INTRODUCTION

Nanotherapeutics employs technologies at the nanoscale, and is one of the most promising routes to control oxidative stress, by inducing apoptosis of damaged cells.¹ Oxidative stress a major factor of aging and life span,² and is caused by the accumulation of highly reactive oxygen species (ROS) due to an insufficient buffering by antioxidant defences.³ ROS oxidize cell constituents such as lipids, proteins and DNA, and compromises cells structures and functions. As such, oxidative stress has been related to chronic inflammations, cancer, and neurodegenerative and immune-deficient disorders.⁴⁻⁵

Nanoceria is a versatile, commercially valuable catalytic material with properties profoundly different from the parent bulk material. Nanoceria's activity can be tuned via synthetic protocol, particle size, nature and level of dopant, particle shape and surface chemistry. Ceria has commercial utility in the areas of chemical mechanical polishing and planarization, catalytic converters and diesel oxidation catalysts, intermediate temperature solid oxide fuel cells and sensors.⁶ Its potential future uses include chemical looping combustion, photolytic and thermolytic water splitting for hydrogen production and as a therapeutic agent for the treatment of certain human diseases.

Among nanotechnologies, nanozymes – metal oxide nanomaterials that display enzyme mimetic activity, have drawn great interest.^{1, 7-9} Nanoceria fits within this class of materials because of its remarkable redox activity arising from the easy conversion between oxidised CeO_2 and oxygen deficient CeO_{2-x} . This property enables nanoceria to act catalytically to capture, store and release oxygen. Nanoceria has long been employed by the catalysis and energy industries,⁶ but only recently was probed in biomedicine,¹⁰⁻²⁰ to protect against radiation-induce cellular damage,²¹ anti-inflammatory²² and antioxidant^{13, 23-24} agents, self-regulated bioassays,²⁵ growth promoter of stem cell,²⁶ protection agent in cardiovascular disease,²⁷⁻²⁸ detection agent of cancer biomarkers,^{29 30} and therapeutic agent for retinal degeneration³¹⁻³² and neuroprotection³³⁻³⁵ including Alzheimer's disease.³⁶

Nanoceria has many and competing enzyme mimetic activities but the most important are those related to the suppression of ROS. At the nanoscale, surface defects, including oxygen vacancies and Ce^{3+} ions, are responsible for the ROS scavenging activity of nanoceria, enabling it to mimic the activity of superoxide dismutase^{14, 37-39} and catalase^{13, 39-43}. Nanoceria exploitation in nanotherapeutics rests with its ability to capture, store and release oxygen as much as in any other catalytic applications,⁶ thus requiring control over those many factors influencing its activity, including particle size and morphology,⁴⁰ $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio, electrolyte species,⁴¹ and pH.^{16, 20, 36} Unlike clinical activity, which is focused on the physiological response of cells in the presence of nanoceria, here we focus on the design of the material to control mimetic activity.

The interaction of nanoceria with electrolyte species, which are abundant in cellular environments, has been largely overlooked, yet they may ‘poison’ the nanoceria catalyst. A common inorganic anion is phosphate, which can interact with nanoceria surfaces and interfere with ROS scavenging.^{41-42, 44-45} However, the mechanisms underpinning phosphorus ‘poisoning’ are unclear, as much as the poisoning and deactivation of catalysts by SO_x are still big challenges in catalysis.⁶ Here, we investigate phosphorus ‘poisoning’ using both first principle and classical modelling. We predict at the atomic level that the interaction is an intricate balance between the surface defect concentration (oxygen vacancies and Ce^{3+}), and hydroxyl groups adsorbed on the nanoceria surfaces. We discuss this in the context of the most suitable nanoceria morphologies for reducing the interference of phosphate with ROS scavenging activity. Such understanding will also provide insight into poisoning of ceria nanocatalysts exploited for other applications, such as clean air and energy materials (fuel cell) technologies.⁶

METHODOLOGY

Quantum Mechanics. Density Functional Theory (DFT) calculations were performed using the Vienna Ab initio Simulation Package (VASP),⁴⁶⁻⁴⁷ within which projector augmented wave pseudopotentials and a plane wave cutoff of 500 eV were used. Calculations were carried out using

the generalized gradient approximation (GGA) exchange-correlation functional of Perdew, Burke and Ernzerhof revised for solids (PBEsol)⁴⁸, with the +U correction of Dudarev⁴⁹ to account for on-site Coulombic interactions. A U value of 5eV is applied to Ce f states.⁵⁰

The structures were geometrically optimized until the residual forces on each atom were less than 10 meV Å⁻¹. All calculations were spin polarized and a ferromagnetic ordering was used throughout, which was shown to bare no difference in the energetic of CeO₂ systems. The details of model structures are provided in Supporting Information.

Classical Mechanics. A model nanoparticle of CeO₂ comprising 18849 atoms was generated using simulated amorphisation and crystallisation, details of this strategy can be found in reference.⁵¹ During crystallisation the structure of the nanoparticle evolves exposing {111}, {110} and {100} surfaces in accord with the real nanomaterial. 628 oxygen vacancies (together with 1256 Ce³⁺ for charge balance) were then randomly introduced into the model nanoparticle and MD simulation was run, at 2000K, for 2.5 ns. This relatively high temperature MD simulation was performed to enable the oxygen vacancies to locate to low energy equilibrium positions within the nanoparticle (including on the nanoparticle surface); the nanoparticle was then cooled. Inspection of the model revealed the presence of oxygen vacancies, which reside throughout the nanoparticle and also on the surface, Figure 1.

Nanoparticle preparation. Bare Nanoceria particles were synthesized according to the procedure described in the paper.^{52 53} Typically, an appropriate amount of Cerium (IV) ammonium nitrate was dissolved in the DI water and the reaction was carried out at 100 °C under reflux conditions with continues stirring. An appropriate amount of ammonium hydroxide was added slowly to the equilibrated solution mixture and the reaction was continued for 24 h to obtain CeO₂ nanoparticle dispersion.

Phosphate Ion Treatment. The phosphate ion buffer solution was prepared by mixing the 1:1 ratio of a 50 mM aqueous solution of Na₂HPO₄ and 50 mM aqueous solution of NaH₂PO₄. The molar ratio

of phosphate ion buffer solution and nanoparticle was maintained as 4:1 and incubated period was 24 h. The treated nanoparticles were separated by multiple centrifugation for XPS analysis.

Characterization. The particle size was measured using High-Resolution Transmission Electron Microscopy (HRTEM) (FEI Technai F30 TEM), at a potential of 300 kV. The selected areas electron diffraction (SAED) pattern was used to analyse the crystallinity of nanoparticles. The analysis of the electronic/vacancy state of nanoceria was completed with the use of an X-ray Photoelectron Spectrophotometer. The Instrument used was a Physical Electronics 5400 ESCA using unmonochromatized Mg K_{α} x (1253.6 eV) ray sources. The experiments were all conducted at ultrahigh vacuum (UHV) with a maximum internal pressure of 5×10^{-8} Torr within the instrument. The spectra observed are comparative standards that we have used to analyse the change in the surface chemistry of cerium, oxygen and phosphorous under their new chemical configuration. The spectra have all been referenced and shifted to the carbon peak position at 284.5 eV in order to compensate for charging due to irradiation.

RESULTS

Nanoceria Morphology. The calculated nanoceria equilibrium morphology is a truncated octahedron expressing {111}, {100} and {110} surfaces, and was first found using simulated amorphization and recrystallization (Figure 1).⁵¹ The surface structure (Figure 1a,c,d,e), surface reduction (Figure 1b),⁵⁴ and surface hydroxylation^{50, 55} all influence the catalytic performance of nanoceria, and are therefore central to our first principle analysis and design of schemes (Figure 2) that we use to disentangle the role of phosphate on nanoceria surfaces. These schemes are constructed to represent possible experimental routes that nanoceria will undergo during cellular uptake and are based on the experimental evidence that phosphate adsorption increases surface Ce^{3+} concentration. Figure 2 also includes the diagrams for the interaction energy of phosphate, which describes the strength of the interaction between the phosphate species and the different nanoceria {111}, {110} and {100} surfaces. The interaction energy is presented for Scheme 1 (nanoceria hydroxylated

oxygen stoichiometric surfaces) as a function of surface concentrations of hydroxyl groups and Ce^{3+} , for Scheme 2 (nanoceria oxygen deficient surfaces) as a function of surface concentrations of oxygen vacancies and Ce^{3+} , and for Scheme 3 (nanoceria hydroxylated oxygen deficient surfaces) as a function of surface concentrations of hydroxyl groups and Ce^{3+} . These Schemes are explained in details hereafter.

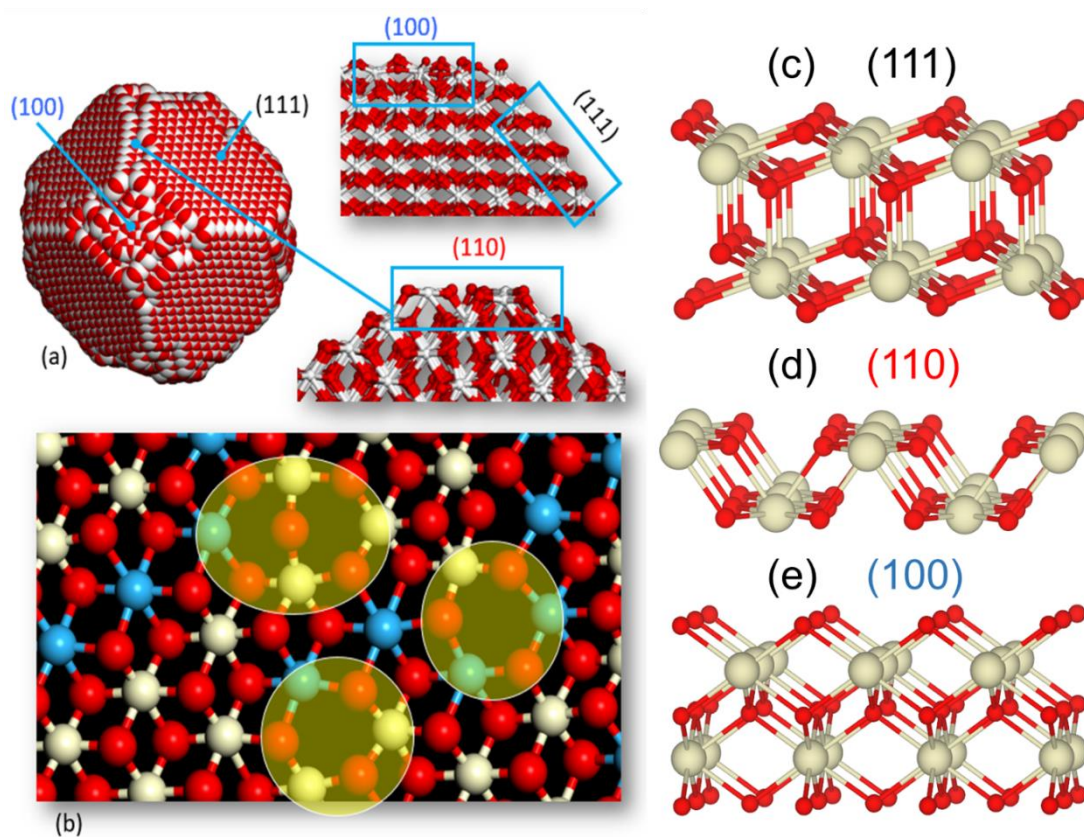


Figure 1. (a) Structure of a nanoparticle of ceria showing {111}, {110} and {100} surfaces. (b) View of one of the nanoparticle's {111} surfaces after nanoceria has been reduced reveals oxygen vacancies residing on the surface as indicated by the yellow ovals. The structure of 'perfect' (c) {111}, (d) {110} and (e) {100} surfaces of nanoceria simulated using DFT are consistent with the structures of the surfaces exposed by the nanoparticle (a). Ce^{4+} is white, Ce^{3+} is blue and oxygen is red.

Scheme 1 – Phosphate adsorption on hydroxylated stoichiometric ceria surfaces. Scheme 1 considers the adsorption of phosphate on hydroxylated, oxygen stoichiometric surfaces of nanoceria

(Figure 2a). In this scenario the ratio of $\text{Ce}^{3+}/\text{Ce}^{4+}$ is governed only by the concentration of surface hydroxyl groups (x_{OH}), and as this increases, the concentration of Ce^{3+} increases.

Figure 2a shows the strength of the interaction between phosphate and nanoceria when its surfaces are hydroxylated. The preferential affinity of phosphates follows the order $\{100\} > \{110\} > \{111\}$ as the more negative energy terms indicate the strongest interaction. We observe that across the majority of our ab initio calculations, phosphate adsorbs with a tridentate geometry (Figure 3a), enabling surface Ce atoms to recover the oxygen coordination, partially on the $\{110\}$ and $\{100\}$, and fully on the $\{111\}$ surface, which explains the order of energetic affinity. The strongest interaction occurs at the $\{100\}$ surface, which undergoes a large surface reconstruction to accommodate the adsorbed phosphate (Figure 3b).

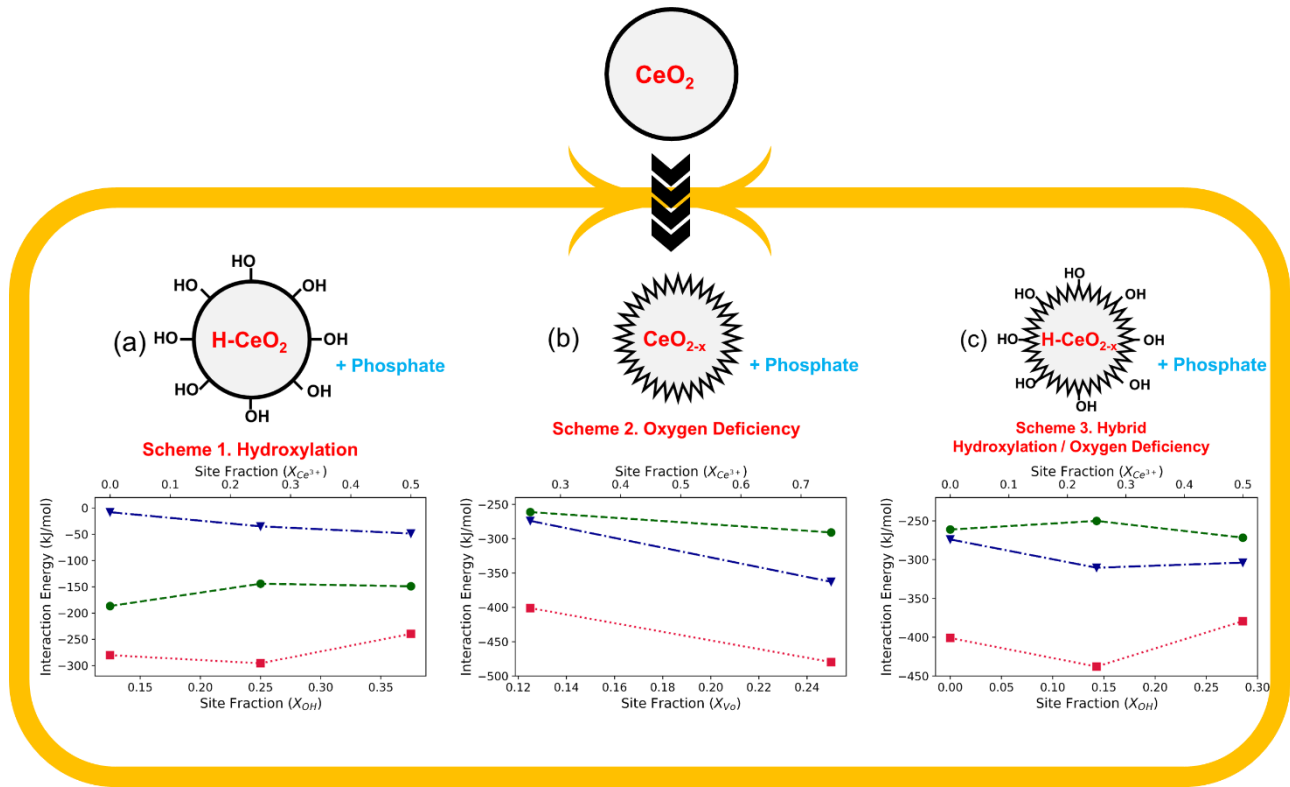


Figure 2. Interaction energy of phosphate with nanoceria for three compositions of nanoceria in a living cell. Interaction energy (kJ/mol) of phosphate at nanoceria surfaces $\{111\}$ (blue triangles), $\{110\}$ (green circles), and $\{100\}$ (red squares), (a) Scheme 1, as a function of coverage of OH and Ce^{3+} on hydroxylated oxygen stoichiometric surfaces; (b) Scheme 2, as a function of coverage of Vo

and Ce^{3+} oxygen deficient surfaces; (c) Scheme 3, as a function of coverage of OH and Ce^{3+} on hydroxylated oxygen deficient surfaces.

Scheme 2 – Phosphate adsorption on surfaces with oxygen vacancies. Scheme 2 considers the adsorption of phosphates on oxygen deficient surfaces of nanoceria by introducing surface oxygen vacancies (Figure 2b). The $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio is thus governed by the oxygen stoichiometry (x_{Vo}), i.e. as the concentration of oxygen vacancies increases so the concentration of Ce^{3+} increases to maintain charge neutrality.

Figure 2b shows the interaction strength between phosphate and oxygen deficient nanoceria. Here, the phosphate interacts strongly and directly with the surface oxygen vacancies by filling the vacant site (Figure 3c). This interaction becomes stronger as the surfaces become more defective (more Ce^{3+} and oxygen vacancies). This mechanism has been proposed experimentally.^{41-42, 44-45, 56-57} When oxygen vacancies are present on the surfaces of nanoceria, our ab initio calculations reveal a barrier-less dissociative adsorption of phosphate whereby a hydrogen adsorbs on a nearby surface oxygen (Figure 3c).

Unlike on hydroxylated surfaces (Scheme 1), the strength of interaction between nanoceria and phosphate in Scheme 2 follows the order $\{100\} > \{111\} > \{110\}$. Although there is certainly a component due to the extent of recovery of surface Ce atoms coordination, this works shows that it also depends on the ease of removal of oxygen from the surface (i.e. heat of reduction), which follows the order $\{111\} > \{100\} > \{110\}$.⁵⁰ The adsorption of phosphate on oxygen deficient surfaces is accompanied by an oxidative reaction of Ce^{3+} to Ce^{4+} . As the $\{111\}$ has a larger energy barrier to reduction (254.72 kJ/mol) compared to the $\{110\}$ surface (177.53 kJ/mol), the $\{111\}$ surface is easier to re-oxidise and hence stabilise upon adsorption of phosphate. The anomaly is still the $\{100\}$ surface, where the heat of reduction lies between the $\{111\}$ and $\{110\}$ surfaces (192.01 kJ/mol). This surface

shows the strongest interaction because of the large surface reconstruction (Figure 4b), which over-stabilizes the surface upon phosphate adsorption.

Scheme 3 – Phosphate adsorption on hydroxylated surfaces with oxygen vacancies. Scheme 3 is a hybrid scenario between Scheme 1 and Scheme 2, where the adsorption of phosphate on nanoceria oxygen deficient surfaces depends on hydroxylation (Figure 2c). The ratio Ce^{3+}/Ce^{4+} is governed by surface hydroxyl groups (x_{OH}) at a constant concentration of surface oxygen vacancies (x_{Vo}).

Similar to Scheme 2, in Scheme 3 (Figure 2c) the preferential affinity of phosphate follows the order $\{100\} > \{111\} > \{110\}$, with phosphate adsorbing directly onto oxygen vacancies displaying a barrier-less dissociation (Figure 3b). We again see a large surface reconstruction of the $\{100\}$ surface of nanoceria, which again makes this surface the most favourable for phosphate adsorption (Figure 3b).

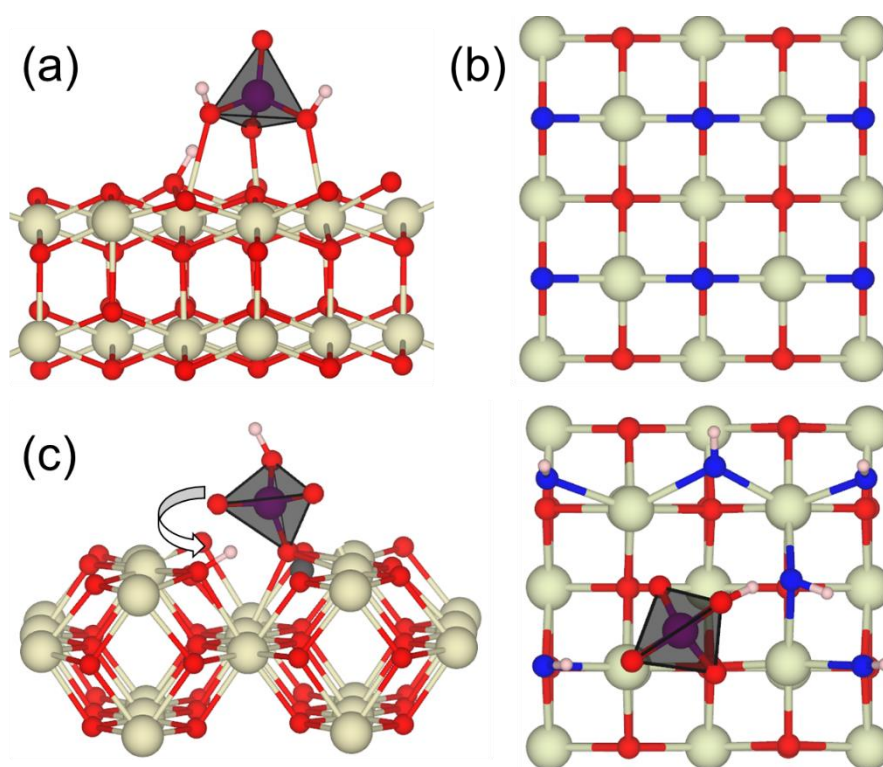


Figure 3. Examples of (a) phosphate adsorbed with a tridentate geometry on the $\{111\}$ surface of nanoceria, (b) reconstruction of the outer oxygen layer on the $\{100\}$ surface of nanoceria when

phosphate is adsorbed, and (c) phosphate directly adsorbed on an oxygen vacancies at the {110} surface of nanoceria associated with a barrier-less dissociation (arrow) whereby a hydrogen adsorbs on a nearby surface oxygen. Ce is white and oxygen is red. Outermost oxygen is blue in (b) to show the surface reconstruction.

Experimental surface analysis of nanoceria after phosphate ion treatment. Details of sample preparation and characterization, and phosphate treatment are available in supporting information. SAED patterns of the 8-10 nm synthesized nanoparticles (Figure 4a), reveal (111), (200), (220) and to a lesser extent (311) lattice planes, confirming a cubic fluorite CeO_2 structure.

To align with the modelling work, Ce^{4+} -rich nanoceria was treated with phosphate and targeted towards early-stage phosphate-ceria interaction; phosphate concentrations were controlled to prevent CePO_4 formation and as in our calculations the adsorption of phosphate promotes formation of Ce^{3+} . The spectra of the cerium 3d, oxygen 1s and the phosphorous 2p of nanoceria particles before and after phosphate buffer treatment was obtained using high resolution XPS (Figure 4(b-d)). Surface cerium ions in nanoceria are mostly Ce^{4+} before (30% Ce^{3+} and 70% Ce^{4+}) and after (43% Ce^{3+} and 57% Ce^{4+}) phosphate treatment at neutral pH, although the Ce^{3+} concentration increased 13% in the treated sample resulting in a Ce^{3+} concentration of 43% (Figure 4b). Ce^{3+} concentrations can increase by as much as 21% with treated nanoceria, resulting in Ce^{3+} concentrations of 80%.⁴⁵ Details of Ce^{4+} and Ce^{3+} concentrations are given in the supporting information.

Analysis of oxygen binding energies and peak structures before and after treatment shows predominant asymmetry for both treated and untreated nanoceria, which is attributed to surface CO (single or double bond formation) and hydroxide (OH^-) species. The absence of a major peak shift in the O1s region of the treated sample indicates that the chemical binding of phosphate oxygen does not change significantly the fluorite structure (i.e. no formation of CePO_4 ⁴⁵). In Figure 4c, the peak at ~529 eV in the emission spectrum is attributed to ceria structural O-Ce bonding and exists in both treated and untreated samples; there is a small shift of this peak at higher binding energy (~0.3 eV)

indicating that the concentration of Ce^{3+} has indeed increased in the treated sample. The intensity of the HO-Ce bonding at ~ 532 eV is increased and broader in the phosphate-treated sample, which indicates that surface hydroxyl groups play a key role in phosphate ion adsorption. The peak at ~ 532 eV has also shifted at higher binding energy (~ 0.3 eV) indicating again that there is a higher concentration of Ce^{3+} (interacting with hydroxyl groups) after the treatment with phosphate. Indeed weakly-bound phosphate interactions can be detected on the surface of nanoceria after treating the material with phosphate buffer solution (Figure 4d), which indicates the nanoparticle surface displays interaction with phosphate due to residual Ce^{3+} oxidation state on its surface. There is now a PO-Ce peak in oxygen binding energies (Figure 4c) after nanoceria has been treated with phosphate; this is seen as a peak at ~ 531.5 eV. As this peak is not as intense as the O-Ce peak in the treated sample, this is proof that CePO_4 has not formed on Ce^{4+} -rich nanoceria unlike for Ce^{3+} -rich nanoceria.⁴⁵

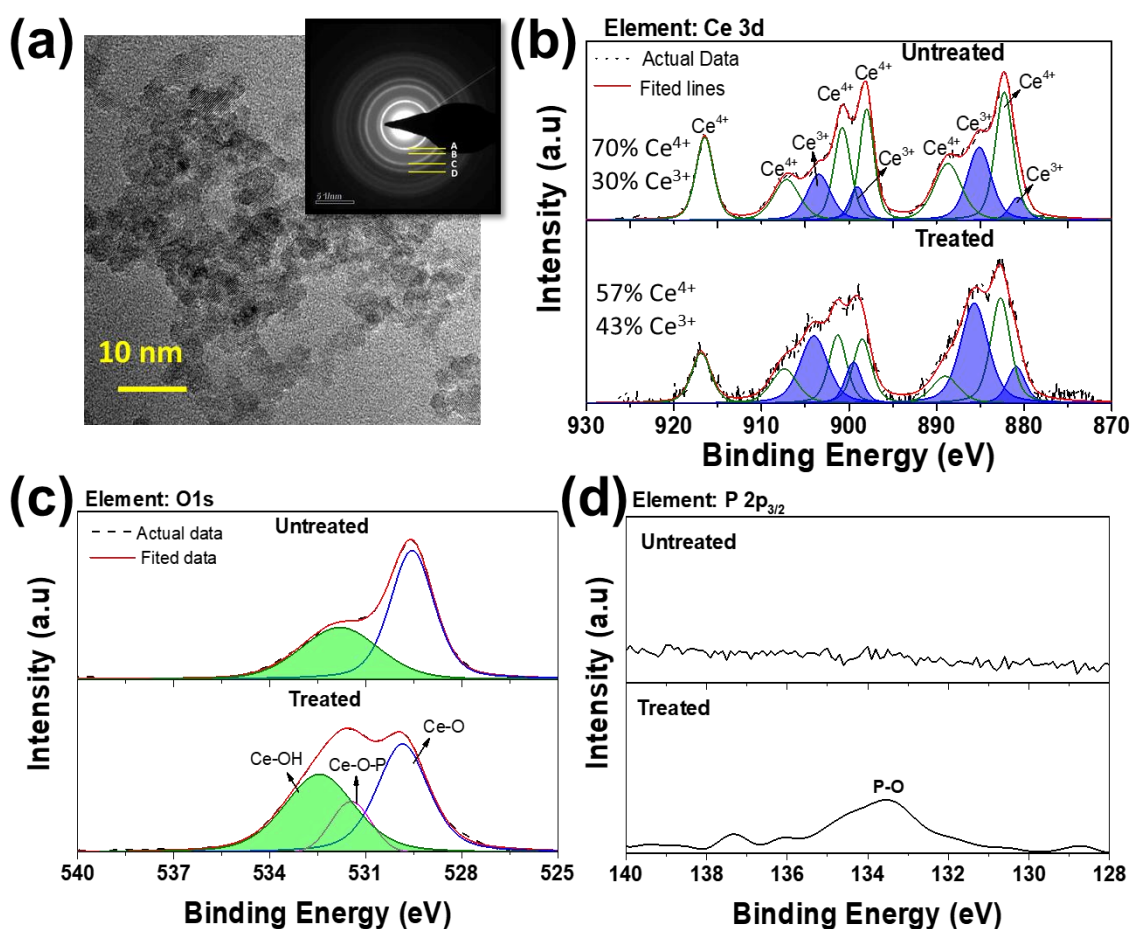


Figure 4. (a) TEM image of nanoceria with SAED patterns and the particle of 5-10 nm range. (A, B, C, D are the 111, 200, 220 and 311 lattice plane. (b-d) High resolution XPS spectra of nanoceria before and after incubation with phosphate. (b) Ce 3d spectra: the concentration of Ce^{3+} is increased after interaction with phosphate ($\sim 13\%$); (c) O 1s spectra: PO-Ce peak has formed in treated sample; (d) P 2p spectra: intense emission in the 2p region was observed after treatment, which indicates the presence of phosphate on the surface of nanoceria sample.

Factors controlling the phosphate interaction with nanoceria. At the molecular level, we predict that there are three factors that contribute to the phosphate-ceria interaction strength.

Surface hydroxyl concentration has a minor effect on the phosphate/ceria interaction strength. In particular for oxygen deficient surfaces with HO- (Scheme 3), and without HO- (Scheme 2), there is a minimal change in interaction energy (Figure 2b and 2c). The hydrogen bonding network seems therefore to have little impact on the adsorption of phosphate on nanoceria.

Surface oxygen vacancy concentration influences considerably the phosphate/ceria interactions. Specifically, the interactions are much stronger when surface oxygen vacancies are present (Scheme 2 and 3) compared to surfaces without oxygen vacancies (Scheme 1). This is regardless of whether the surface is hydroxylated (Scheme 3) or not (Scheme 2).

Surface Ce^{3+} concentration surprisingly does not increase the phosphate/ceria interaction. In particular the interaction energy remains fairly ‘flat’ as Ce^{3+} increases (Scheme 1 and Scheme 3). The only case that we observe a stronger interaction, as Ce^{3+} concentration increases, is on surfaces that are oxygen deficient but not hydroxylated (Figure 2b – Scheme 2). Finally we note that phosphate usually adsorbs on the surfaces maximising its interaction with Ce^{3+} .

An unusual reconstruction. Of particular note is phosphate adsorption on the $\{100\}$ surface (Figure 3b). In particular, the surface oxygen layer on the $\{111\}$ and $\{110\}$ surfaces remains highly ordered when phosphate is adsorbed. Conversely, adsorption on the $\{100\}$ surface results in a large

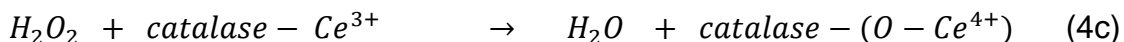
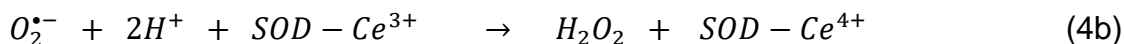
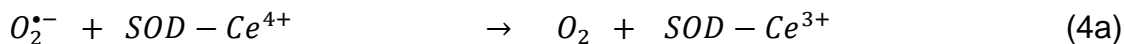
reconstruction, which maximises the bonding network between surface cerium and phosphate. This reconstruction appears for all {100} surfaces, whether hydroxylated or oxygen deficient (hence in all our schemes studied). The reconstruction is likely due to the flexibility of the {100} surface oxygen network, which can easily access many different surface oxygen arrangements due to very small energy differences between these arrangements, and thus this surface can easily accommodate adsorbed species, which further stabilizes the surface.^{58 59} This is the first time that evidence of this behaviour has been found for the adsorption of inorganic anions (i.e. phosphate).

DISCUSSION

Implication for nanotherapeutics. Like for any application of nanoceria, the biomedical interpretation at the molecular level of the therapeutic action of nanoceria as a potentially important nanozyme requires an understanding of the surface structure and composition, but this time its modification in the context of biologically relevant environments. In this study, we have shown how the presence of phosphate, a common bodily electrolyte, strongly adsorbs and modifies the surfaces of nanoceria, which as noted, it can mitigate oxidative stress, by buffering some of the most reactive oxygen species (ROS) in cellular environments. This is similar to the poisoning of ceria nanocatalysts exploited or unwanted for other ceria based technologies, whether for energy materials or catalysts.⁶ Although, the mechanisms of reaction of nanoceria with ROS within the cellular environment are somewhat obscure, it is generally accepted that the scavenging of these reactive species follows Scheme 4; superoxide dismutase (SOD) interacts with the superoxide radical $O_2^{\bullet-}$ ^{14, 37-39} (Scheme 4a,b) and catalase with hydrogen peroxide H_2O_2 ^{13, 39-43} (Scheme 4c,d). In analogy, similar mechanisms of scavenging are likely to underpin ‘clean-air’ technologies (i.e. water splitting and three way catalysis), exploiting the oxygen storage capacity of ceria.⁶

From a modeling viewpoint, although still in its infancy, first principles simulation can unravel the interaction of radicals with nanoceria.⁶⁰⁻⁶³ However, this approach for exploring the mechanisms controlling enzyme mimetic activities, requires detailed knowledge of the species present at the

surfaces. Hence, in this work we targeted the poisoning effect of phosphate on the selective exploitation of nanoceria nanozyme activities.



Scheme 4. Physiological reactions of ROS in the presence of nanoceria. (1) Superoxide dismutase. It is a disproportionation reaction where Ce active sites react with two superoxide ions: one is oxidized to molecular oxygen (eqn (4a)) and the other is reduced to hydrogen peroxide (eqn (4b)). (2) Catalase. It is a disproportionation reaction where Ce active sites react with two hydrogen peroxide molecules: one is reduced to water (eqn (4c)) and the other is oxidized to water and molecular oxygen (eqn (4d)).

Experimental work has shown that the highest superoxide scavenging activity is exhibited by Ce^{3+} -rich nanoceria,³⁸ which is active in both neutral and acidic environments,²⁰ and is retained upon adsorption of some biomolecules and polymers.³⁷ Conversely, Ce^{4+} -rich nanoceria has stronger catalase activity, which appears to be independent of morphology, i.e. nanorods and nanocubes.⁴⁰ Exposure to phosphate⁴⁵ poisons SOD activity,⁶⁴ but not catalase activity.^{41, 44} It is thus clear that the presence of phosphate has a selective impact on the enzyme activity.

The simulation results are also supported by XPS, as the spectra (Figure 4a) show that Ce^{3+} concentration increases upon phosphate adsorption, we can infer that there is indeed a preferential driving force for phosphate to interact with Ce^{3+} active sites at the surface of nanoceria; indeed Ce^{3+} -rich nanoceria has even been shown to undergo structural reconstruction with the $CePO_4$ phase forming.⁴⁵ This demonstrates that any enzyme mimetic activity that is catalysed by Ce^{3+} , i.e. SOD,

will be inhibited by the presence of phosphate due to steric sequestration of the reactive sites. The strong affinity of phosphate to Ce^{3+} will also block the conversion to Ce^{4+} , quenching the redox activity of nanoceria. Experimentation supports this, as unlike Ce^{3+} -rich nanoceria, Ce^{4+} -rich nanoceria does not show a strong affinity for phosphate; CePO_4 does not form on Ce^{4+} -rich nanoceria.^{41 42 44 45 56 57} The interaction of phosphate with nanoceria also appears to be dependent upon pH (i.e. HO^- concentration) but only for small nanoparticles.⁵⁶ Specially, a change in pH results in a change in the surface composition in terms of the concentration of adsorbed hydroxyl species. Our data indicates that there is a variation (sometimes small) in the interaction between phosphate and nanoceria when the concentration of hydroxyl groups increases, but that this is dependent on the surface morphology and oxygen composition (Figure 2a/Scheme 1 and Figure 2c/Scheme 3). Of note is the {100} surface, which shows a less favourable interaction when the concentration of hydroxyl groups increases independently of surface composition (Figure 2a and 2c). Here, our data is predictive because experiment has not yet focused on morphological effects. The experiments also support the *ab initio* data indicating that hydroxyl groups help the phosphate-nanoceria interaction as demonstrated by the change in relative intensity of the Ce-OH and Ce-O peaks after phosphate treatment (Figure 4c). However, the interaction between phosphate and hydroxyl groups appears to be marginal compared to the interaction between Ce^{3+} and phosphate (Figure 2), as the strength of interaction shown by our modelling data is independent on the increase in concentration of OH groups (Scheme 1 and Scheme 3).

Our modelling data support the experimental evidence (Figure 4c-d) that there is a strong affinity of nanoceria with phosphate - particularly on the {100} surface because of a heavy surface reconstruction (Figure 3b) irrespective of whether the surface is oxygen stoichiometric (Figure 2a – Scheme 1) or deficient (Figure 2c – Scheme 3) (Figure 3b). Ceria nanocubes will therefore experience a greater reduction of SOD enzyme mimetic activity in the presence of phosphate, which is independent of surface composition (Scheme 1, 2 or 3). On the other hand, ceria nano-octahedra with

a high concentration of hydroxyl groups, will offer greater resistance to phosphate adsorption, thus limiting the quenching of SOD activity; the interaction energy is the weakest and only a small amount of energy would be needed to remove the phosphate (Figure 2a – Scheme 1).

It is worth noting that our data can only shed light on the early stage of phosphate adsorption. In high phosphate concentration, Ce^{3+} -rich nanoceria transforms in cerium phosphate (CePO_4), unlike Ce^{4+} -rich nanoceria in this study.⁴¹ The precipitation of CePO_4 was seen *in vitro*^{41-42, 44-45} and in root cells of cucumber plants, where cerium accumulated both as CeO_2 and CePO_4 .⁶⁵⁻⁶⁶ Future modelling may target the formation of CePO_4 by evolving nanoceria surfaces exposed to phosphate.

The interaction of nanoceria with phosphates is also seen in its phosphatase mimetic activity; this is the hydrolysis of the phosphoester bonds. Although we do not aim at proposing a mechanism from *ab initio* calculations, we consider only the activation of nanoceria surface due to the interaction with phosphate. The nucleophilic substitution seems to be the more plausible mechanism underpinning the phosphatase activity, i.e. dephosphorylation of ATP and other organophosphates.⁶⁷ Dephosphorylation depends upon the availability of Ce^{3+} ,⁵⁷ although high oxygen vacancy and low hydroxyl group concentrations on the surface reduce the phosphatase activity;⁶⁸ the activation of phosphorus to nucleophilic attack requires surface hydroxyl groups acting as nucleophilic agents.

Our *ab initio* calculations show that the interaction of phosphate with nanoceria surfaces is stronger when the surfaces are both hydroxylated and oxygen deficient (Figure 2c – Scheme 3) compared to when they are only hydroxylated (Figure 2a – Scheme 1). Specially, phosphate adsorbs directly on oxygen vacancies (Figure 3c). Such surface anchoring facilitates nucleophilic attack of phosphate with hydroxyl group (nucleophile).⁶⁹ Dephosphorylation was reported to be pH independent,⁷⁰ but morphology dependent.⁶⁷ Our data shows a great variation in the interaction energies within the different surfaces supporting the morphology dependent adsorption of phosphate with the most favourable interactions exhibited by the $\{100\}$ oxygen stoichiometric (Figure 2a – Scheme 1) or deficient (Figure 2c – Scheme 3) surfaces. The $\{111\}$ surface is also a good candidate for anchoring

the phosphate, but only when the surface is both hydroxylated and oxygen deficient (Figure 2c – Scheme 3). We can also infer from our experiments that phosphate and Ce^{3+} concentrations cannot be too high at the surface of nanoparticles as nanoceria will otherwise transform into CePO_4 , thus inhibiting the phosphatase activity. Our modelling, also support the experimentation (Figure 4c) as it sees hydroxyl groups playing an important role in adsorbing phosphates (Figure 2a), although this depends strongly on the presence of oxygen vacancies, which are needed to stabilize the phosphate adsorption at the surface and facilitate the nucleophilic attack (Figure 2c); this is again morphology dependent.

Although phosphatase mimetic activity of nanoceria is an exciting discovery, there are currently no concrete biological applications. One explanation for this might be that the ceria nanomaterial was not optimised and therefore its phosphatase mimetic activity not detected experimentally. Accordingly, we predict that to maximise phosphatase mimetic activity, nanoceria should be synthesised with truncated octahedral or cuboidal morphology, to maximise the CeO_2 {100} surface exposure, together with Ce^{3+} , and a high HO- concentration (Figure 2c – Scheme 3).

CONCLUSIONS

The exploitation of nanoceria enzyme mimetic activity depends strongly on the nanoscale structure and chemistry of its surfaces. This is as important for any biomedical application of nanoceria as for any other energy technologies.

Density Functional Theory modelling has provided atomistic insights into the strength of phosphate poisoning, an important bodily ion that can hinder the biological exploitation of nanoceria enzyme mimetic activity. It is indeed likely that the effects shown here, also provide insights into the catalytic processes and poisoning (i.e. sulfur poisoning) in ‘clean-air’ or fuel-cell technologies as the ceria nanomaterial will more likely undergo the same mechanisms, i.e. oxygen capture, storage and release, exploited in each technology.

Oxygen deficient nanoceria interact strongly with phosphate compared to hydroxylated nanoceria. However this interaction depends strongly on surface structure. Specially, whether nanoceria consist of {111}, {110} or {100} surfaces.

As the strongest interaction occurs between {100} surfaces and phosphate due to a large surface reconstruction, our data suggest that ceria nanocubes bind phosphate strongly, which will inhibit $\text{Ce}^{4+}/\text{Ce}^{3+}$ redox and nanozyme activity to a greater extent compared to ceria nano-octahedra comprising {111} surfaces, as these display much weaker interactions with phosphate.

Our simulation provides a prescription for high phosphatase mimetic activity – the nanomaterial should have polyhedral or cuboidal morphology to maximise exposure of CeO_2 {100} surfaces and comprise a high concentration of Ce^{3+} and oxygen vacancies; the pH should be adjusted in preparation of the nanotherapeutic, or encapsulated to maintain a local environment (*in vivo*), to provide a high HO^- concentration.

There is room here for investigating other aspects to this work, notably whether the interaction of phosphate and nanoceria is affected by the presence of ligands and polymers for biocompatible coating, and other biomolecules characteristic of biological media. However this should constitute the object of further work.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI:XXXX.

Details of computational and experimental methodologies.

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Notes

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